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Polyphenol oxidase from Pectobacterium atrosepticum: identification and cloning of gene and characteristics of the enzyme

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Abstract

© 2017 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim In the present study, we attempted to elucidate if the harmful phytopathogenic bacteria of Pectobacterium genus (P. atrosepticum) possess the enzymes for oxidation of phenolic compounds. Polyphenol oxidase (laccase) activity was revealed in P. atrosepticum cell lysates. Using bioinformatic analysis, an ORF encoding a putative copper-containing polyphenol oxidase of 241 amino acids with a predicted molecular mass of 25.9 kDa was found. This protein (named Pal1) shares significant level of identity with laccases of a new type described for several bacterial species. Cloning and expression of the pal1 gene and the analysis of corresponding recombinant protein confirmed that Pal1 possessed laccase activity. The recombinant Pal1 protein was characterized in terms of substrate specificity, kinetic parameters, pH and temperature optimum, sensitivity to inhibitors and metal content. Pal1 demonstrated alkali- and thermo-tolerance. The kinetic parameters K m and kcat for 2,6-dimethoxyphenol were 0.353 ± 0.062 mM and 98.79 ± 4.9 s −1 , respectively. The protein displayed high tolerance to sodium azide, sodium fluoride, NaCl, SDS and cinnamic acid. The transcript level of the pal1 gene in P. atrosepticum was shown to be induced by plant-derived phenolic compound (ferulic acid) and copper sulfate.

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Keywords

laccase, Pectobacterium atrosepticum, polyphenol oxidase

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